

AMENDMENT TO THE CLAIMS

1. (Previously Presented) An analytical kit comprising:
 - i) an analytical device comprising a passage allowing a liquid to flow through the same, formed by bonding together a first member having a groove, 1 μ m to 5 mm width and 1 μ m to 750 μ m depth in cross-section, and a second member covering the groove, and a first nucleic acid (N1) having an arbitrary base sequence and immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;
 - ii) a reagent A containing (1) a conjugate (N2-L1) composed of a second nucleic acid (N2) having a sequence at least complementary to the base sequence of the first nucleic acid and (2) a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed;
 - iii) a reagent B containing a conjugate (L2-M) resulting from binding of a marker (M) to a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed.
2. (Previously Presented) An analytical kit comprising:
 - i) an analytical device comprising a passage allowing a liquid to flow through the same, formed by bonding together a first member having a groove, 1 μ m to 5 mm width and 1 μ m to 750 μ m depth in cross-section, and a second member covering the groove, and a first nucleic acid (N1) having an arbitrary base sequence and immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;

- ii) a reagent A containing a conjugate (N2-L1) composed of (1) a second nucleic acid (N2) having a sequence at least complementary to the base sequence of the first nucleic acid and (2) a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed;
- iii) a reagent B' containing a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed; and
- iv) a reagent C containing a conjugate (L3-M) composed of a third ligand (L3) capable of specifically binding to the second ligand (L2) and a marker (M).

3. (Previously Presented) An analytical kit containing no marker and comprising:
 - i) an analytical device comprising a passage allowing a liquid to flow through the same, formed by bonding together a first member having a groove, 1 μm to 5 mm width and 1 μm to 750 μm depth in cross-section, and a second member covering the groove, and a first nucleic acid (N1) having an arbitrary base sequence and immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together; and
 - ii) a reagent A containing a conjugate (N2-L1) composed of (1) a second nucleic acid (N2) having a sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone of the analytical device and (2) a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed.

4. (Previously Presented) An analytical kit comprising:

- i) an analytical device comprising a passage allowing a liquid to flow through the same formed by bonding together a first member having a groove, 1 μm to 5 mm width and 1 μm to 750 μm depth in cross-section, and a second member covering the groove, a first nucleic acid (N1) having an arbitrary base sequence and immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, and a conjugate (N2-L1) composed of a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed and a second nucleic acid (N2) having a base sequence at least complementary to the immobilized first nucleic acid and immobilized in the capturing zone in the form of a conjugate (N1-N2-L1) by specific binding between the first nucleic acid (N1) and second nucleic acid (N2); and
- ii) a reagent B containing a conjugate (L2-M) resulting from binding between a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed and a marker (M).

5. (Currently Amended) An analytical kit comprising the reagent B', reagent C and analytical device specified below in combination:

- i) an analytical device comprising a passage allowing a liquid to flow through the same, formed by bonding together a first member having a groove, 1 μm to 5 mm width and 1 μm to 750 μm depth in cross-section, and a second member covering the groove, a first nucleic acid (N1) having an arbitrary base sequence and immobilized in

a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, and a conjugate (N2-L1) composed of a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed and a second nucleic acid (N2) having a base sequence at least complementary to the immobilized first nucleic acid (N1) and immobilized in the capturing zone in the form of a conjugate (N1-N2-L1) by specific binding between the first nucleic acid (N1) and second nucleic acid (N2); and

- ii) a reagent B' containing a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed; and
- iii) a reagent C containing a conjugate (L3-M) composed of a third ligand (L3) capable of specifically binding to the second ligand (L2) and a marker (M).

6. (Previously Presented) An analytical kit comprising:

- i) an analytical device comprising a passage allowing a liquid to flow through the same, formed by bonding together a first member having a groove, 1 μm to 5 mm width and 1 μm to 750 μm depth in cross-section, and a second member covering the groove, and a plurality of first nucleic acid species (N1g: g being an integer), each having an arbitrary base sequence and immobilized independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;
- ii) a reagent A solution containing a plurality of conjugate species (N2h-L1i: h and i each independently being an integer), each composed of (1) one of a plurality of second nucleic acid species (N2h: h being an integer) and each having a sequence at

least complementary to the base sequence of one of the plurality of first nucleic acid species and (2) one of a plurality of first ligand species (L1i: i being an integer) which is capable of specifically binding to the corresponding one of biological substance species (Ok: k being an integer) to be assayed; and

iii) a reagent B containing conjugate species (L2j-Ml: j and l each independently being an integer) resulting from binding between one or more second ligand species (L2j: j being an integer) capable of specifically binding to corresponding one of biological substance species to be assayed and one or more marker species (Ml: l being an integer).

7. (Previously Presented) An analytical kit comprising:

- i) an analytical device comprising a passage allowing a liquid to flow through the same, formed by bonding together a first member having a groove, 1 μ m to 5 mm width and 1 μ m to 750 μ m depth in cross-section, and a second member covering the groove, and a plurality of first nucleic acid species (N1g: g being an integer) each having an arbitrary base sequence and immobilized independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;
- ii) a reagent A solution containing a plurality of conjugate species (N2h-L1i: wherein h and i are integers, each composed of (1) one of a plurality of second nucleic acid species (N2h: h being an integer), each having a sequence at least complementary to the base sequence of one of the plurality of first nucleic acid species and (2) one of a plurality of first ligand species (L1i: i being an integer) which is capable of

specifically binding to one of biological substance species (Ok: k being an integer) to be assayed;

- iii) a reagent B' containing one or more second ligand species (L2j: j being an integer), each capable of specifically binding to one of the one or more biological substance species to be assayed; and
- iv) a reagent C containing conjugate species (L3m-Ml: wherein m and 1 are integers composed of one or more third ligand species (L3m: m being an integer) capable of specifically binding to a corresponding one of the one or more second ligand species and one or more marker species (Ml: l being an integer).

8. (Previously Presented) An analytical kit comprising:

- i) an analytical device comprising a passage allowing a liquid to flow through the same and formed by bonding together a first member having a groove, 1 μ m to 5 mm width and 1 μ m to 750 μ m depth in cross-section, and a second member capable of covering the groove, and a plurality of first nucleic acid species (N1g: g being an integer), each having an arbitrary base sequence and immobilized independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;
- ii) a reagent A containing a plurality of conjugate species (N2h-L1i: h and i each independently being an integer), each composed of one of a plurality of second nucleic acid species (N2h: h being an integer), each having a sequence at least complementary to the base sequence of the corresponding one of the plurality of first

nucleic acid species and one of a plurality of first ligand species (L1i: i being an integer) which is capable of specifically binding to a corresponding one of biological substance species (Ok: k being an integer) to be assayed.

9. (Previously Presented) An analytical kit comprising the reagent B and analytical device specified below in combination:

- i) an analytical device comprising a passage allowing a liquid to flow through the same and formed by bonding together a first member having a groove, 1 μm to 5 mm width and 1 μm to 750 μm depth in cross-section, and a second member covering the groove, and a plurality of first nucleic acid species (N1g: g being an integer), each having an arbitrary base sequence and immobilized independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, and conjugate species (N2h-L1i: wherein h and i are each an integer), each composed of one of a plurality of first ligand species (L1i: i being an integer), which is capable of specifically binding to a corresponding one of biological substance species (Ok: k being an integer) to be assayed, and one of a plurality of second nucleic acid species (N2h: h being an integer) and which has a base sequence at least complementary to one of the first nucleic acid species immobilized in the capturing zone in the form of conjugate species (N1g-N2h-L1i: g, wherein h and i are each an integer) by specific binding between the first nucleic acid species and second nucleic acid species; and
- ii) a reagent B containing conjugate species (L2j-M1: wherein j and l are each an integer) resulting from binding between one or more second ligand species (L2j: j

being an integer) respectively capable of specifically binding to the corresponding one biological substance species to be assayed and one or more marker species (M₁: 1 being an integer).

10. (Previously Presented) An analytical kit comprising:

i) an analytical device comprising a passage allowing a liquid to flow through the same, formed by bonding together a first member having a groove, 1 μ m to 5 mm width and 1 μ m to 750 μ m depth in cross-section, and a second member covering the groove, a plurality of first nucleic acid species (N_{1g}: g being an integer), each having an arbitrary base sequence and immobilized independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, and conjugate species (N_{2h}-L_{1i}: h and i each independently being an integer), each composed of one of a plurality of first ligand species (L_{1i}: i being an integer), which is capable of specifically binding to a corresponding one of the one or more biological substance species (O_k: k being an integer) to be assayed, and one of a plurality of second nucleic acid species (N_{2h}: h being an integer), which has a base sequence at least complementary to a corresponding one of the immobilized first nucleic acid species (N_{1g}: g being an integer), each conjugate species (N_{2h}-L_{1i}) independently immobilized in the capturing zone in the form of conjugate species (N_{1g}-N_{2h}-L_{1i}: wherein g, h and i are each an integer) by specific binding between the first nucleic acid species and second nucleic acid species; and

- ii) a reagent B' containing one or more second ligand species (L2j: j being an integer) capable of specifically binding to a corresponding one of the biological substance species to be assayed;
- iii) a reagent C containing conjugate species (L3m-Ml: wherein m and l are each an integer) derived from one or more third ligand species (L3m: m being an integer) capable of specifically binding to corresponding one of the second ligand species (L2j: j being an integer) and one or more marker species (Ml: l being an integer).

11. (Original) An analytical kit according to any of Claims 1 to 10, wherein the biological substance(s), first ligand(s) (L1 or L1i: i being an integer), second ligand(s) (L2 or L2j: j being an integer) and/or third ligand(s) (L3 or L3m: m being an integer) is/are selected from among immunological substances, receptors, receptor-binding substances, sugars, glycoproteins, glycolipids, lectins and nucleic acids.

12. (Original) An analytical kit according to Claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, wherein the first ligand or ligands (L1 or L1i: i being an integer) and/or second ligand or ligands (L2 or L2j: j being an integer) are different in reactivity.

13. (Original) An analytical kit according to Claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, wherein the first ligand or ligands (L1 or L1i: i being an integer) and/or second ligand or ligands (L2 or L2j: j being an integer) are identical in reactivity.

14. (Original) An analytical kit according to any of Claims 1 to 10, wherein the marker or markers (M or M₁: 1 being an integer) each is selected from among enzymes, colloidal metals, latexes, nucleic acids, luminescent substances, fluorescent substances, intercalators, biotin, avidin and streptavidin.

15. (Canceled)

16. (Canceled)

17. (Canceled)

18. (Previously Presented) An analytical device comprising a passage allowing a liquid to flow through the same, formed by bonding together a first member having a groove, 1 μ m to 5 mm width and 1 μ m to 750 μ m depth in cross-section, and a second member covering the groove to form the passage, a first nucleic acid (N₁) having an arbitrary base sequence immobilized in a capturing zone provided in the passage prior to bonding the first member and second member together, and a conjugate (N₂-L₁) composed of a first ligand (L₁) capable of specifically binding to a biological substance (O) to be assayed and a second nucleic acid (N₂) having a base sequence at least complementary to the immobilized first nucleic acid (N₁) and immobilized in the capturing zone by specific binding between the first nucleic acid (N₁) and second nucleic acid (N₂).

19. (Previously Presented) An analytical device comprising a passage allowing a liquid to flow through the same formed by bonding together a first member having a groove, 1 μm to 5 mm width and 1 μm to 750 μm depth in cross-section, and a second member covering the groove to form the passage, a plurality of first nucleic acid species (N1g: g being an integer), each having an arbitrary base sequence and immobilized independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, conjugate species (N2h-L1i: h and i each being an integer), each conjugate species being composed of one of a plurality of a first ligand species (L1i: i being an integer), which is capable of specifically binding to a corresponding one of biological substance species (Ok: k being an integer) to be assayed, and one of a plurality of second nucleic acid species (N2h: h being an integer), each of which has a base sequence at least complementary to a corresponding one of the immobilized first nucleic acid species and which is immobilized independently, from species to species, in the capturing zone by specific binding between the first nucleic acid species and second nucleic acid species.

20. (Canceled)

21. (Canceled)

22. (Canceled)

23. (Cancelled)

24. (Previously Presented) An analytical method comprising:

preparing an analytical device, comprising a passage allowing a liquid to flow through the same, by bonding together a first member having a groove, 1 μm to 5 mm width and 1 μm to 750 μm depth in cross-section, and a second member covering the groove;

immobilizing a first nucleic acid (N1), having an arbitrary base sequence, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;

preparing a reagent A containing a conjugate (N2-L1) resulting from binding of a first ligand (L1), capable of specifically binding to a biological substance to be assayed, to a second nucleic acid (N2) having a sequence at least complementary to the base sequence of the first nucleic acid (N1);

mixing a liquid sample suspected of containing the biological substance to be assayed and the reagent A, either after conjugate formation or while allowing conjugate formation to form a mixture;

introducing the mixture into the passage in the analytical device to immobilize the conjugate within the passage; and

assaying the immobilized conjugate.

25. (Previously Presented) An analytical method comprising:

preparing an analytical device, comprising a passage allowing a liquid to flow through the same, by bonding together a first member having a groove, 1 μm to 5 mm width and 1 μm to 750 μm depth in cross-section, and a second member covering the groove;

immobilizing a first nucleic acid (N1), having an arbitrary base sequence, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;

preparing a reagent A containing a conjugate (N2-L1) resulting from binding of a first ligand (L1), capable of specifically binding to a biological substance to be assayed, to a second nucleic acid (N2) having a sequence at least complementary to the base sequence of the first nucleic acid (N1);

separately introducing a liquid sample suspected to contain the biological substance to be assayed and the reagent A, without preliminary mixing of the liquid sample and reagent A, into the passage in the analytical device to immobilize the conjugate within the passage; and

assaying the immobilized conjugate.

26. (Previously Presented) An analytical method comprising:

preparing an analytical device, comprising a passage allowing a liquid to flow through the same, by bonding together a first member having a groove, 1 μm to 5 mm width and 1 μm to 750 μm depth in cross-section, and a second member covering the groove;

immobilizing a plurality of first nucleic acid species (N1g: g being an integer), each having an arbitrary base sequence, independently from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;

preparing a reagent A containing a plurality of conjugate species (N2h-L1i: h and i each being an integer), each resulting from binding of (1) one of a plurality of first ligand species (L1i: i being an integer), capable of specifically binding to a corresponding one of biological substance species (O_k: k being an integer) to be assayed, to (2) one of a plurality of second nucleic acid species (N2h: h being an integer), each having a sequence at least complementary to the base sequence of a corresponding one of the plurality of first nucleic acid species;

mixing a liquid sample, suspected of containing one or more of the biological substance species to be assayed, and the reagent A to form a mixture;

introducing the mixture, either after conjugate formation or while allowing conjugate formation, into the passage in the analytical device to immobilize one or more of the conjugate species within the passage; and

assaying the immobilized conjugate(s).

27. (Previously Presented) An analytical method comprising:

preparing an analytical device, comprising a passage allowing a liquid to flow through the same, by bonding together a first member having a groove, 1 μ m to 5 mm width and 1 μ m to 750 μ m depth in cross-section, and a second member covering the groove;

immobilizing a plurality of first nucleic acid species (N_{1g}: g being an integer), each having an arbitrary base sequence, independently from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;

preparing a reagent A containing a plurality of conjugate species (N_{2h}-L_{1i}: h and i each being an integer) each resulting from binding of (1) one of a plurality of first ligand species (L_{1i}: i being an integer), capable of specifically binding to one of biological substance species (O_k: k being an integer) to be assayed, to (2) one of a plurality of second nucleic acid species (N_{2h}: h being an integer), each having a sequence at least complementary to the base sequence of a corresponding one of the plurality of first nucleic acid species;

separately introducing (1) a liquid sample suspected of containing one or more of the biological substances to be assayed and (2) the reagent A into the passage in the analytical device to immobilize the resulting one or more conjugate species within the passage; and

assaying the immobilized conjugate(s).

28. (Previously Presented) An analytical method using an analytical kit according to claim 1, the method comprising:

mixing two or more of the following materials a, b and c, either after conjugate formation or while allowing conjugate formation, to form a mixture;

a. a liquid sample suspected of containing a biological substance (O) to be assayed,

b. the reagent A containing the conjugate (N2-L1),

c. the reagent B containing the conjugate (L2-M);

introducing the mixture into the passage in the analytical device contained in the analytical kit, followed by introduction of the remaining material of a, b and c, if any, into the passage;

allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone in the analytical device and the second nucleic acid (N2), specific binding between the first ligand (L1) and biological substance (O) and specific binding between the second ligand (L2) and biological substance (O); and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-M).

29. (Previously Presented) An analytical method using an analytical kit according to claim 1, the method comprising:

separately introducing the following materials a, b and c given below individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

a. a liquid sample suspected of containing a biological substance (O) to be assayed,

b. the reagent A containing a conjugate (N2-L1),

c. the reagent B containing the conjugate (L2-M);

allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone and the second nucleic acid (N2), specific binding between the first ligand (L1) and biological substance (O) and specific binding between the second ligand (L2) and biological substance (O); and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-M).

30. (Previously Presented) An analytical method using the analytical kit according to claim 2, the method comprising:

mixing two or more of the following materials a, b, c and d, either after conjugate formation or while allowing conjugate formation, to form a mixture:

- a. a liquid sample suspected of containing a biological substance (O) to be assayed,
- b. the reagent A containing a conjugate (N2-L1),
- c. the reagent B' containing a second ligand (L2), and
- d. the reagent C containing a conjugate (L3-M);

introducing the mixture into the passage in the analytical device contained in the analytical kit, followed by introduction of the remaining material or materials a, b, c and d, if any, into the passage;

allowing formation of an immobilized conjugate (N1-N2-L1-O-L2-L3-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone and the second nucleic acid (N2), specific binding between the first ligand (L1) and

the biological substance (O), specific binding between the second ligand (L2) and the biological substance (O) and specific binding between the second ligand (L2) and third ligand (L3); and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-L3-M).

31. (Previously Presented) An analytical method using the analytical kit according to claim 2, the method comprising:

separately introducing the following materials a, b, c and d individually, without any mixing, into the passage in the analytical device contained in the analytical kit:

- a. a liquid sample suspected of containing a biological substance (O) to be assayed,
- b. the reagent A containing the conjugate (N2-L1),
- c. the reagent B' containing the second ligand (L2), and
- d. the reagent C containing the conjugate (L3-M);

allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-L3-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone and the second nucleic acid (N2), specific binding between the first ligand (L1) and biological substance (O), specific binding between the second ligand (L2) and biological substance (O) and specific binding between the second ligand (L2) and third ligand (L3); and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-L3-M).

32. (Previously Presented) An analytical method using the analytical kit according to claim 2, the method comprising:

preparing a marker-carrying biological substance (O-M) in advance from a liquid sample suspected of containing a biological substance (O) to be assayed by introduction of a marker (M) into that substance;

introducing the reagent A containing the conjugate (N2-L1), either after conjugate formation or while allowing conjugate formation, into the passage in the analytical device contained in the analytical kit;

allowing the formation of an immobilized conjugate (N1-N2-L1-O-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone and the second nucleic acid (N2); and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-M).

33. (Previously Presented) An analytical method using the analytical kit according to claim 2, the method comprising:

preparing a marker-carrying biological substance (O-M) in advance from a liquid sample suspected of containing a biological substance (O) to be assayed by introduction of a marker (M) into that substance;

separately introducing (1) the reagent A containing the conjugate (N2-L1) assayed and (2) the marker-carrying biological substance (O-M) individually, without mixing together, into the passage in the analytical device contained in the analytical kit;

allowing the formation of an immobilized conjugate (N1-N2-L1-O-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone and the second nucleic acid (N2); and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-M).

34. (Previously Presented) An analytical method using the analytical kit according to claim 4, the method comprising:

mixing the following materials a and b to form a mixture:

- a. a liquid sample suspected of containing a biological substance (O) to be assayed,
- b. the reagent B containing the conjugate (L2-M);

introducing the mixture, either after conjugate formation or while allowing conjugate formation, into the passage in the analytical device;

allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-M) by specific binding between the first ligand (L1) in the conjugate (N1-N2-L1) immobilized in the capturing zone and the biological substance (O) and by specific binding between the second ligand (L2) in the conjugate (L2-M) and the biological substance (O); and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-M).

35. (Previously Presented) An analytical method using an analytical kit according to claim 4, the method comprising:

separately introducing the following materials a and b individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

a. a liquid sample suspected of containing a biological substance (O) to be assayed,

b. the reagent B containing the conjugate (L2-M);

allowing formation of an immobilized conjugate (N1-N2-L1-O-L2-M) by specific binding between the first ligand (L1) in the conjugate (N1-N2-L1) immobilized in the capturing zone in the analytical device and the biological substance (O) and by specific binding between the second ligand (L2) in the conjugate (L2-M) and the biological substance (O); and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-M).

36. (Previously Presented) An analytical method using an analytical kit according to claim 5, the method comprising:

mixing two or more of the following materials a, b and c to form a mixture;

- a. a liquid sample suspected of containing the biological substance (O) to be assayed,
- b. the reagent B', and,
- c. the reagent C containing the conjugate (L3-M);

introducing the mixture, either after further conjugate formation or while allowing further conjugate formation, into the passage in the analytical device, followed by introduction of the remaining material a, b or c, if any, into the passage; allowing further conjugate formation to produce an immobilized conjugate (N1-N2-L1-O-L2-L3-M) by specific binding between the first ligand (L1) in the conjugate (N1-N2-L1) immobilized in the capturing zone and the biological substance (O), specific binding between the second ligand (L2) and biological substance (O) and specific binding between the second ligand and third ligand; and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-L3-M).

37. (Previously Presented) An analytical method using an analytical kit according to claim 5, the method comprising:

introducing the following materials a, b and c individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

- a. a liquid sample suspected of containing a biological substance (O) to be assayed,
- b. the reagent B' containing the second ligand (L2)
- c. the reagent C containing the conjugate (L3-M);

allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-L3-M) by specific binding between the first ligand (L1) in the conjugate (N1-N2-L1) immobilized in the capturing zone and the biological substance (O), specific binding between the second ligand (L2) and biological substance (O) and specific binding between the second ligand and third ligand; and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-L3-M).

38. (Previously Presented) An analytical method using an analytical kit according to claim 6, the method comprising:

mixing two or more of the following materials a, b and c to form a mixture:

- a. a liquid sample suspected of containing one or more biological substance species (Ok: k being an integer) to be assayed,
- b. the reagent A solution containing conjugate species N2h-L1i, and;
- c. the reagent B containing conjugate species L2j-Ml;

introducing the mixture, either after conjugate formation or while allowing conjugate formation, into the passage in the analytical device contained in the analytical kit, followed by further introduction of the remaining material a, b, and c, if any, into the passage;

allowing the formation of conjugate species (N1g-N2h-L1i-Ok-L2j-Ml: wherein g, h, i, j, k and l are each an integer), each immobilized independently, from species to species, by specific binding between the plurality of first nucleic acid species and the plurality of second nucleic acid species, specific binding between the

plurality of first ligand species and the one or more biological substance species and specific binding between the one or more second ligand species and the one or more biological substance species; and

assaying the one or more biological substance species by detecting the one or more marker species contained in the plurality of immobilized conjugate species.

39. (Previously Presented) An analytical method using an analytical kit according to claim 6, the method comprising:

introducing the following materials a, b and c individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

- a. a liquid sample suspected of containing one or more biological substance species (Ok: k being an integer) to be assayed,
- b. the reagent A solution containing the conjugate species N2h-L1i,
- c. the reagent B containing the conjugate species L2j-M1;

allowing the formation of conjugate species (N1g-N2h-L1i-Ok-L2j-M1: wherein g, h, i, j, k and l are each integer) immobilized each independently, from species to species, by specific binding between the plurality of first nucleic acid species immobilized independently, from species to species, in the capturing zone and the plurality of second nucleic acid species (N2h), specific binding between the plurality of first ligand species (L1i) and the one or more biological substance species and specific binding between the one or more second ligand species and the one or more biological substance species;

assaying the one or more biological substance species by detecting the one or more marker species (M1) contained in the plurality of immobilized conjugate species N1g-N2h-L1i-Ok-L2j-M1.

40. (Previously Presented) An analytical method using the analytical kit according to claim 7, the method comprising:

mixing two or more of the following materials a, b, c and d to form a mixture:

- a. a liquid sample suspected of containing one or more biological substance species (Ok: k being an integer) to be assayed,
- b. the reagent A solution containing conjugate species N2h-L1i, ,
- c. the reagent B' containing one or more second ligand species L2j, and
- d. the reagent C containing conjugate species L3m-M1;

introducing the mixture into the passage in the analytical device, followed by introduction of the remaining materials a, b, c and d, if any, into the passage;

allowing the formation of conjugate species (N1g-N2h-L1i-Ok-L2j-L3m-M1: wherein g, h, i, j, k, l and m are each an integer), each immobilized independently, from species to species, by specific binding between the plurality of first nucleic acid species and the plurality of second nucleic acid species, specific binding between the plurality of first ligand species and the one or more biological substance species, specific binding between the one or more second ligand species and the one or more biological substance species and specific binding between the one or more second ligand species and the one or more third ligand species;

assaying the one or more biological substance species by detecting the one or more marker species contained in the plurality of immobilized conjugate species (N₁g-N₂h-L₁i-Ok-L₂j-L₃m-Ml: wherein g, h, i, j, k, l and m are each an integer).

41. (Previously Presented) An analytical method using the analytical kit according to claim 7, the method comprising:

introducing the following materials a, b, c and d individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

- a. a liquid sample suspected of containing one or more of the biological substance species to be assayed,
- b. the reagent A solution containing conjugate species N₂h-L₁i,
- c. the reagent B' containing one or more of the second ligand species L₂j, and
- d. the reagent C containing conjugate species L₃m-Ml; and

allowing the formation of conjugate species N₁g-N₂h-L₁i-Ok-L₂j-L₃m-Ml (wherein g, h, i, j, k, l and m are each an integer), each immobilized independently, from species to species, by specific binding between the plurality of first nucleic acid species and the plurality of second nucleic acid species, specific binding between the plurality of first ligand species and the one or more biological substance species, specific binding between the one or more second ligand species and the one or more biological substance species and specific binding between the one or more second ligand species and the one or more third ligand species;

assaying the one or more biological substance species by detecting the one or more marker species contained in the plurality of immobilized conjugate species N1g-N2h-L1i-Ok-L2j-L3m-Ml).

42. (Previously Presented) An analytical method using the analytical kit according to claim 8, the method comprising:

preparing at least one marker-carrying biological substance species Ok-Ml: (wherein k and l are each an integer) from a liquid sample suspected of containing one or more of the biological substance species by introduction of one or more marker species Ml (l being an integer) into the liquid sample;

introducing the reagent A containing conjugate species N2h-L1i into the passage in the analytical device contained in the analytical kit;

allowing formation of conjugate species N1g-N2h-L1i-Ok-Ml (wherein g, h, i, k and l are each being an integer), immobilized each independently, from species to species, by specific binding between the plurality of first nucleic acid species and the plurality of second nucleic acid species and specific binding between the plurality of first ligand species and the at least one biological substance species;

assaying the at least one biological substance species by detecting the one or more marker species contained in the plurality of immobilized conjugate species N1g-N2h-L1i-Ok-Ml.

43. (Previously Presented) An analytical method using the kit according to claim 8, the method comprising:

preparing one or more marker-carrying biological substance species from a liquid sample suspected of containing one or more biological substance species by introduction of one or more marker species M_l (l being an integer) into the liquid sample;

introducing the reagent A containing conjugate species N_{2h}-L_{1i} into the passage in the analytical device contained in the analytical kit;

allowing the formation of conjugate species N_{1g}-N_{2h}-L_{1i}-O_k-M_l (wherein g, h, i, k and l are each an integer), each immobilized independently, from species to species, by specific binding between the plurality of first nucleic acid species immobilized in the capturing zone and the plurality of second nucleic acid species and specific binding between the plurality of first ligand species and the one or more biological substance species; and

assaying the one or more biological substance species by detecting the one or more marker species contained in the plurality of immobilized conjugate species N_{1g}-N_{2h}-L_{1i}-O_k-M_l.

44. (Previously Presented) An analytical method using the analytical kit according to claim 9, the method comprising:

mixing the following materials a and b to form a mixture:

- a. a liquid sample suspected of containing the one or more biological substance species,
- b. the reagent B;

introducing the mixture into the passage in the analytical device contained in the analytical kit;

allowing the formation of conjugate species $N1g-N2h-L1i-Ok-L2j-Ml$ (wherein g, h, i, j, k and l are each an integer), immobilized each independently, from species to species, by specific binding between the plurality of first ligand species in the conjugate species $N1g-N2h-L1i$ and the one or more biological substance species and specific binding between the one or more second ligand species in the conjugate species $L2j-Ml$; and

assaying the one or more biological substance species by detecting the one or more marker species contained in the plurality of immobilized conjugate species $N1g-N2h-L1i-Ok-L2j-Ml$.

45. (Previously Presented) An analytical method using the analytical kit according to claim 9, the method comprising:

introducing the following materials a and b individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

- a. a liquid sample suspected of containing the one or more biological substance species,
- b. a reagent B containing conjugate species $L2j-Ml$;

allowing the formation of conjugate species $N1g-N2h-L1i-Ok-L2j-Ml$ (wherein g, h, i, j, k and l are each an integer), each immobilized independently, from species to species, by specific binding between the plurality of first ligand species in the conjugate species $N1g-N2h-L1i$ and the one or more biological substance species

and specific binding between the one or more second ligand species in the conjugate species L2j-M1 and the one or more biological substance species; and

assaying the one or more biological substance species by detecting the one or more marker species contained in the plurality of immobilized conjugate species N1g-N2h-L1i-Ok-L2j-M1.

46. (Previously Presented) An analytical method using the analytical kit according to claim 10, the method comprising:

mixing two or more of the following materials a, b and c, either after conjugate formation or while allowing conjugate formation, to form a mixture;

- a. a liquid sample suspected of containing the one or more biological substance species to be assayed,
- b. the reagent B' containing one or more second ligand species L2j,
- c. the reagent C containing the conjugate species L3m-M1;

introducing the mixture into the passage in the analytical device followed by introduction of the remaining material a, b and c, if any, into the passage;

allowing the formation of immobilized conjugate species N1g-N2h-L1i-Ok-L2j-L3m-M1 (wherein g, h, i, j, k, l and m are each an integer) by specific binding between the first ligand species L1i in the conjugate species N1g-N2h-M1i immobilized in the capturing zone in the analytical device and the biological substance species Ok, specific binding between the second ligand species L2j and the biological substance species Ok and specific binding between the second ligand species L2j and the third ligand species L3m;

assaying the one or more biological substance species Ok by detecting the one or more marker species Ml contained in the immobilized conjugate species N1g-N2h-L1i-Ok-L2j-L3m-Ml.

47. (Previously Presented) An analytical method using the analytical kit according to claim 10, the method comprising:

introducing the following materials a, b and c individually, without mixing together, into the passage in the analytical device:

- a. a liquid sample suspected of containing one or more of the biological substance species Ok,
- b. the reagent B' containing the one or more second ligand species L2j,
- c. the reagent C containing the conjugate species L3m-Ml;

allowing the formation of immobilized conjugate species N1g-N2h-L1i-Ok-L2j-L3m-Ml (wherein g, h, i, j, k, l are each an integer) by specific binding between the first ligand species L1i in the conjugate species N1g-N2h-Mli and the biological substance species Ok, specific binding between the second ligand species L2j and the biological substance species Ok and specific binding between the second ligand species L2j and the third ligand species L3m; and

assaying the one or more biological substance species Ok by detecting the one or more marker species Ml contained in the immobilized conjugate species N1g-N2h-L1i-Ok-L2j-L3m-Ml.

48. (Previously Presented) An analytical method using the analytical kit according to claim 18, the method comprising the following elements:

preparing in advance a marker-carrying biological substance O-M from a liquid sample suspected of containing a biological substance (O) by introduction of a marker (M) thereinto;

introducing the marker-carrying biological substance O-M into the passage in the analytical device;

allowing the formation of an immobilized conjugate N1-N2-L1-O-M by specific binding between the first ligand L1 in the conjugate L1-N2 immobilized in the capturing zone and the biological substance (O) in the marker-carrying biological substance O-M; and

assaying the biological substance (O) by detecting the marker (M) contained in the immobilized conjugate N1-N2-L1-O-M.

49. (Previously Presented) An analytical method using the analytical kit according to claim 19, the method comprising:

preparing in advance one or more marker-carrying biological substance species Ok-Ml (wherein k and l are each an integer) from a liquid sample suspected of containing one or more of the biological substance species Ok by introduction of one or more markers Ml (l being an integer) thereinto;

introducing the marker-carrying biological substance species Ok-Ml into the passage in the analytical device;

allowing the formation of immobilized conjugate species N₁g-N₂h-L₁i-Ok-M₁ (wherein g, h, i, k and l are each an integer) by specific binding between the plurality of first ligand species L₁i immobilized in the capturing zone and the one or more marker-carrying biological substance species Ok-M₁; and
assaying the one or more biological substance species Ok by detecting the one or more marker species M₁ contained in the immobilized conjugate species N₁g-N₂h-L₁i-Ok-M₁.

50. (Canceled)

51. (Previously Presented) A method of preparing an analytical device comprising:

preparing a first member having a groove, 1 μ m to 5 mm width and 1 μ m to 750 μ m depth in cross-section, and a second member capable of covering the groove, wherein the groove forms a portion of a passage upon joining the first member and second member together and one of the first member and second member or both have a passage inlet and a passage outlet,

immobilizing a nucleic acid (N), having an arbitrary base sequence, at a site on a portion of the first member and/or second member forming the passage, to form a zone for capturing a biological substance to be assayed,

then joining the first member and second member together by thermal fusion or with an adhesive to give an assembly with the passage formed therein,

introducing into the passage a reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a base sequence at least complementary to the base sequence of the first nucleic acid (N1) which is immobilized in the capturing zone and a first ligand (L1) capable of specifically binding to a biological substance to be assayed, and

allowing the conjugate (N2-L1) to specifically bind, for immobilization thereof, to the first nucleic acid (N1) in the capturing zone.

52. (Previously Presented) A method of preparing an analytical device comprising:

preparing a first member having a groove, 1 μm to 5 mm width and 1 μm to 750 μm depth, and a second member capable of covering the groove, wherein the groove forms a portion of a passage upon joining the first member and second member together and one of the first member and second member or both have a passage inlet and a passage outlet,

immobilizing a plurality of first nucleic acid species (N1g: g being an integer) each having an arbitrary base sequence at independent sites forming a zone within the passage for capturing one or more biological substance species to be assayed,

then joining the first member and second member together by thermal fusion or with an adhesive to give an assembly with the passage formed therein,

introducing into the passage a reagent A containing conjugate species (N2h-L1i: wherein h and i are each an integer), each composed of one of a plurality of

second nucleic acid species (N2h: h being an integer), each second nucleic acid species having a base sequence at least complementary to the base sequence of a corresponding species of the plurality of first nucleic acid species (N1g: g being an integer) immobilized in the capturing zone, and one of a plurality of first ligand species (L1i: i being an integer), each first ligand species being capable of specifically binding to a corresponding species of the one or more biological substance species to be assayed , and

allowing the plurality of conjugate species N2h-L1i to specifically bind, for immobilization thereof, to the plurality of first nucleic acid species previously immobilized in the capturing zone.

53. (Canceled)

54. (Original) A method of preparing analytical devices as set forth in Claim 51 or 52, wherein the biological substance or substances and/or first ligand (L1) or ligands are selected from among immunological substances, receptors and nucleic acids.

55. (Canceled)

56. (Canceled)

57. (Canceled)